Figures of the Molecular Characterization of Epigenetic Mutants in *Arabidopsis thaliana:*

Categorization of active *Athila6* elements in *ddm1* and *ddm1/rdr6* mutants

By

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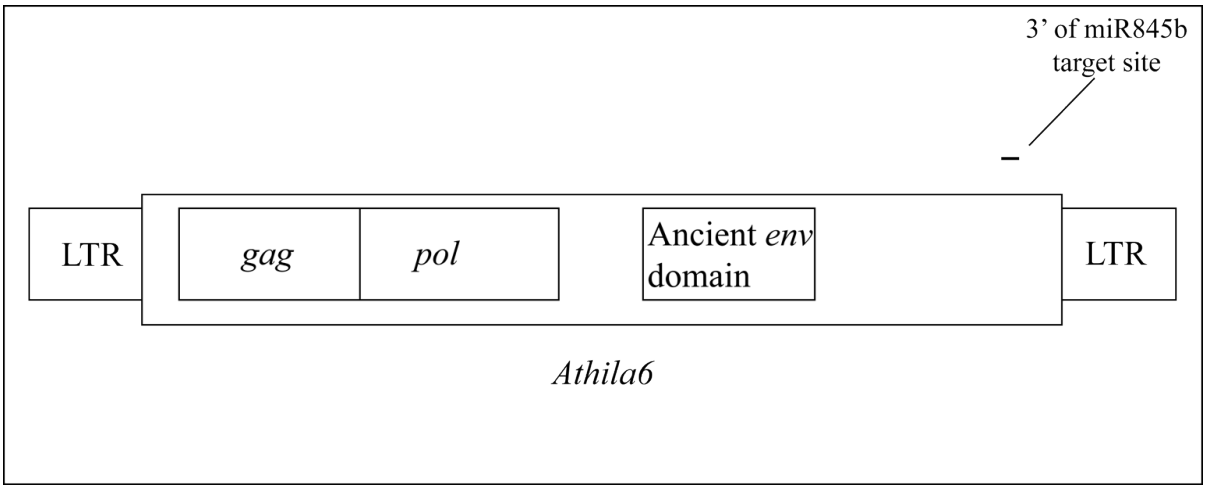

Figure 1- The structure of the *Athila6* LTR retrotransposon. Long Terminal Repeats (LTRs) flank both ends of the element. The LTRs are recognized by the transposition machinery encoded by the *gag* and *pol* genes. The ancient *env* domain is the non-functional remains of an open reading frame. Towards the end of the element there is believed to be a target site for microRNA 845b but this site is not found in all *Athila6* elements.

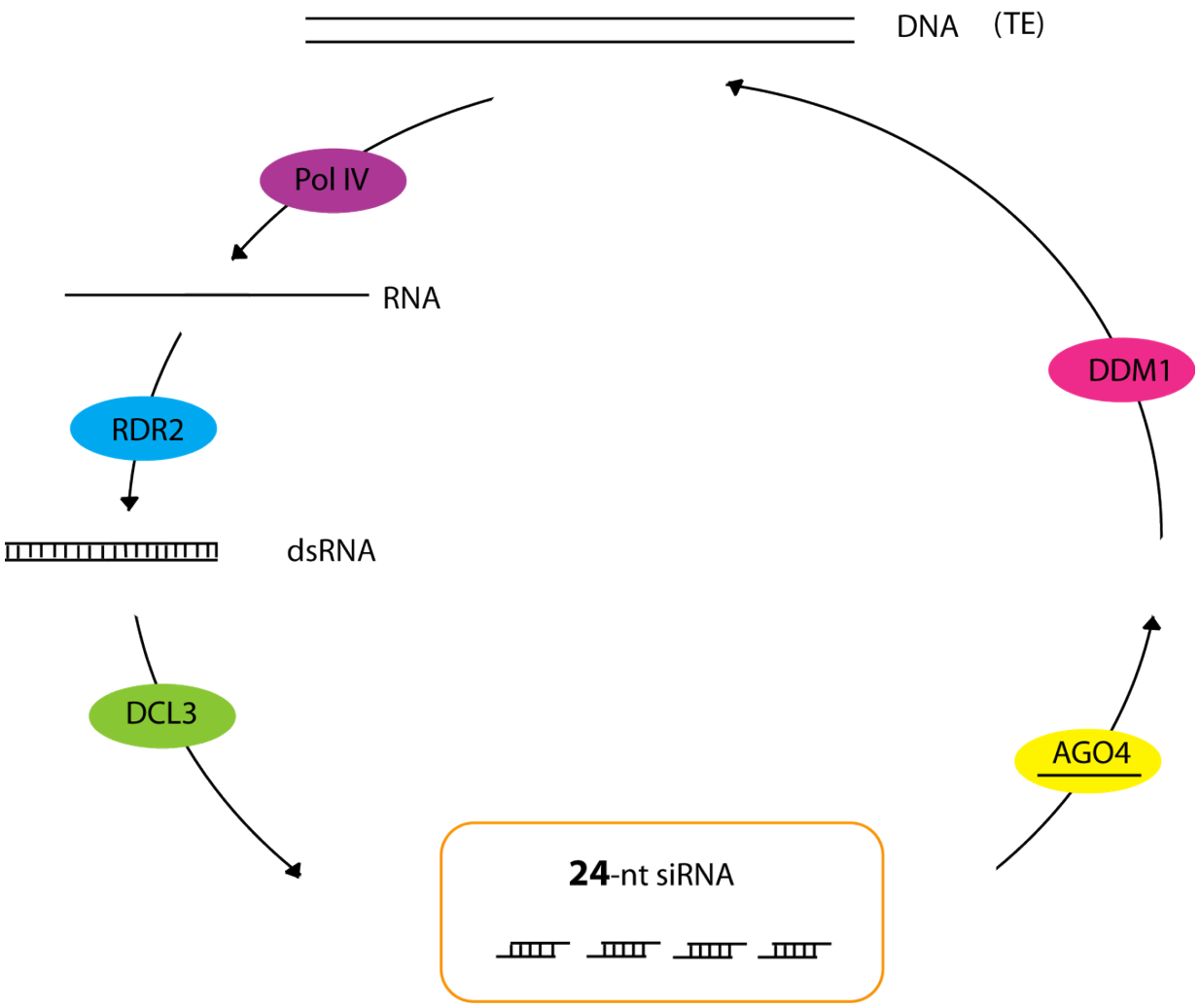

Figure 2– A diagram of the generation of 24-nt siRNA by PolIV, RDR2 and DCL3. This diagram is specific to *Arabidopsis*. Figure created of Andrea McCue and edited by Jennifer Bosse.

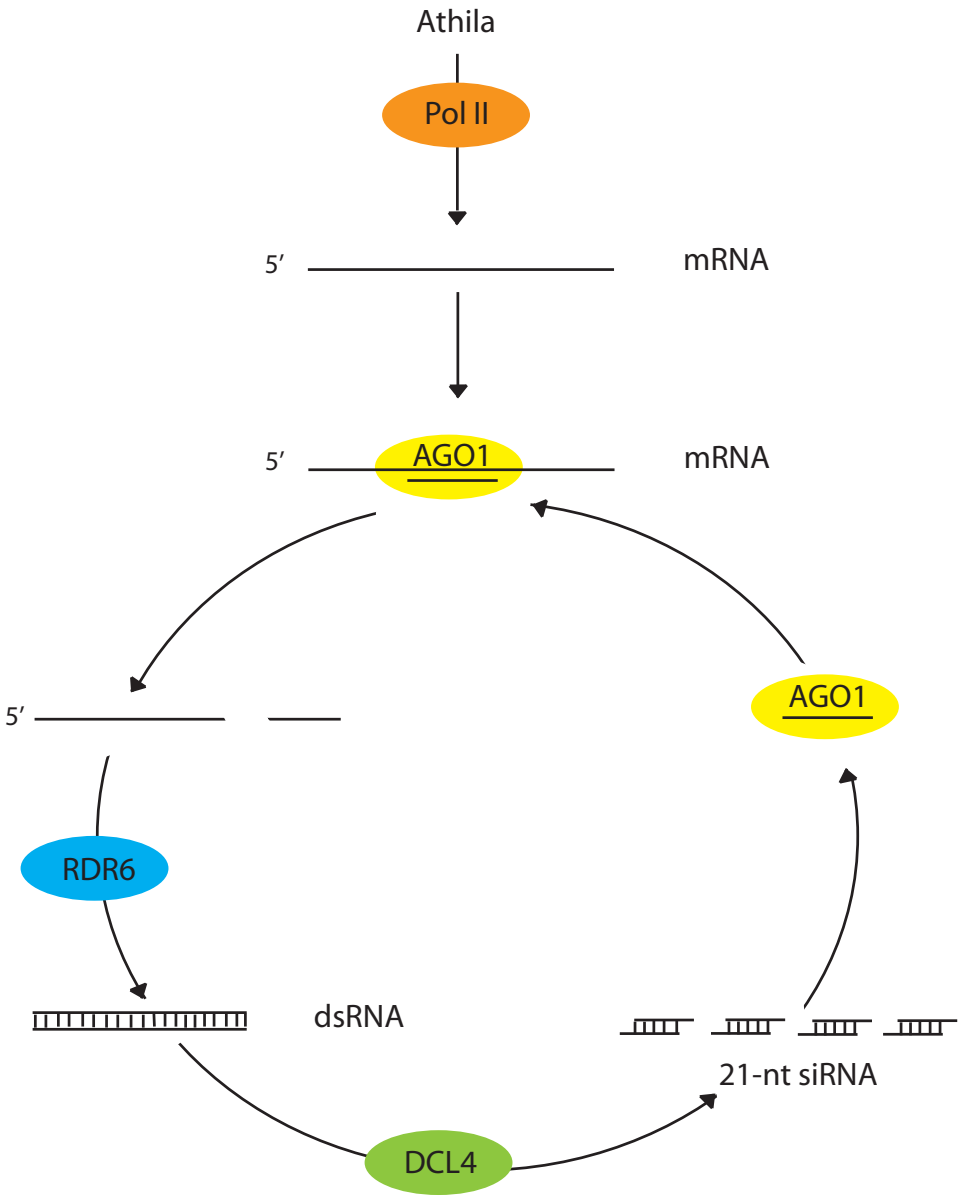

Figure 3– A diagram of the generation of 21-nt siRNA by RDR6 and DCL4. This diagram is Arabidopsis specific. Figure created by Andrea McCue and edited by Jennifer Bosse.

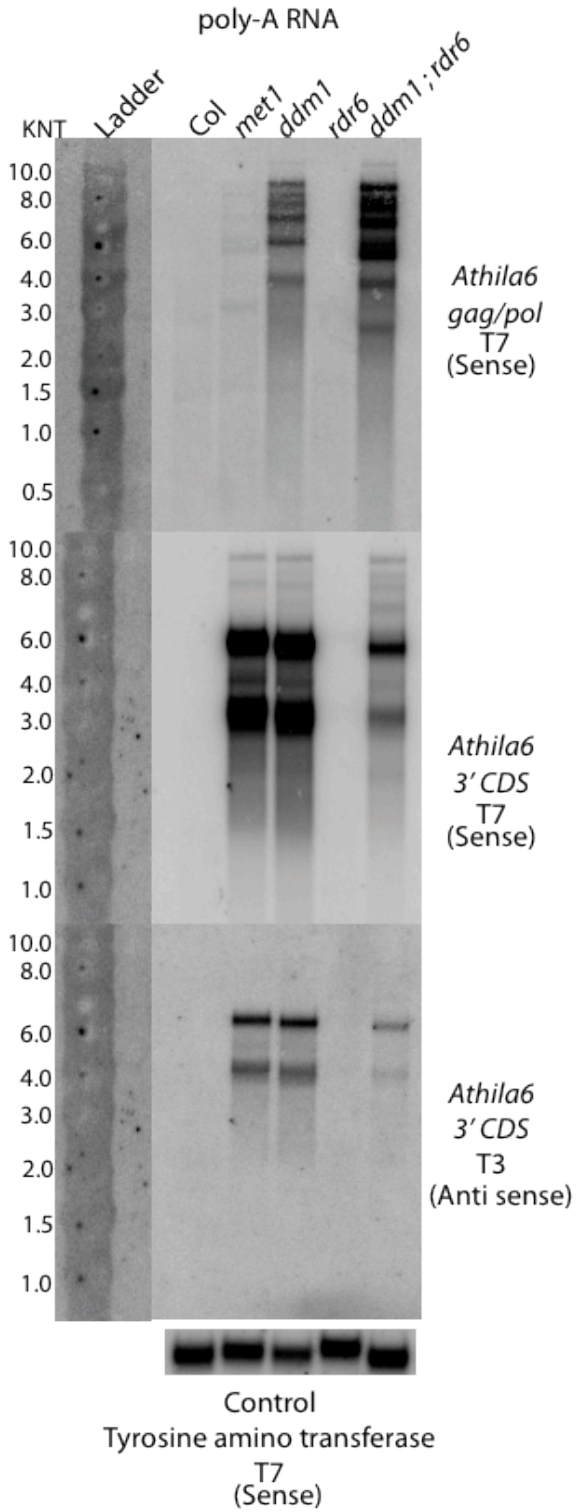

Figure 4- Northern blot analysis of *Athila6* expression in epigenetic mutants, performed by Saivageethi Nuthikattu. The genotypes used in this Northern blot are Col (wild type *Arabidposis)*, *met1(methyltransferase 1* and an epigenetic mutant not studied in this project), *ddm1, rdr6* and *ddm1/rdr6*. The blot was probed with sense *Athila6 gag/pol* T7, sense *Athila6* 3'CDS T7 and anti sence *Athila6* 3'CDS T3. When probed with *Athila6 gag/pol* T7, the *ddm1/rdr6* mutant has around 2.5 KNT and 6 KNT sized transcripts not seen in *ddm1*. When probed with *Athila6* 3'CDS T7 *ddm1/rdr6* mutant has around 7 KNT sized transcripts not seen in *ddm1*. These transcripts will be referred to as the additional transcripts.

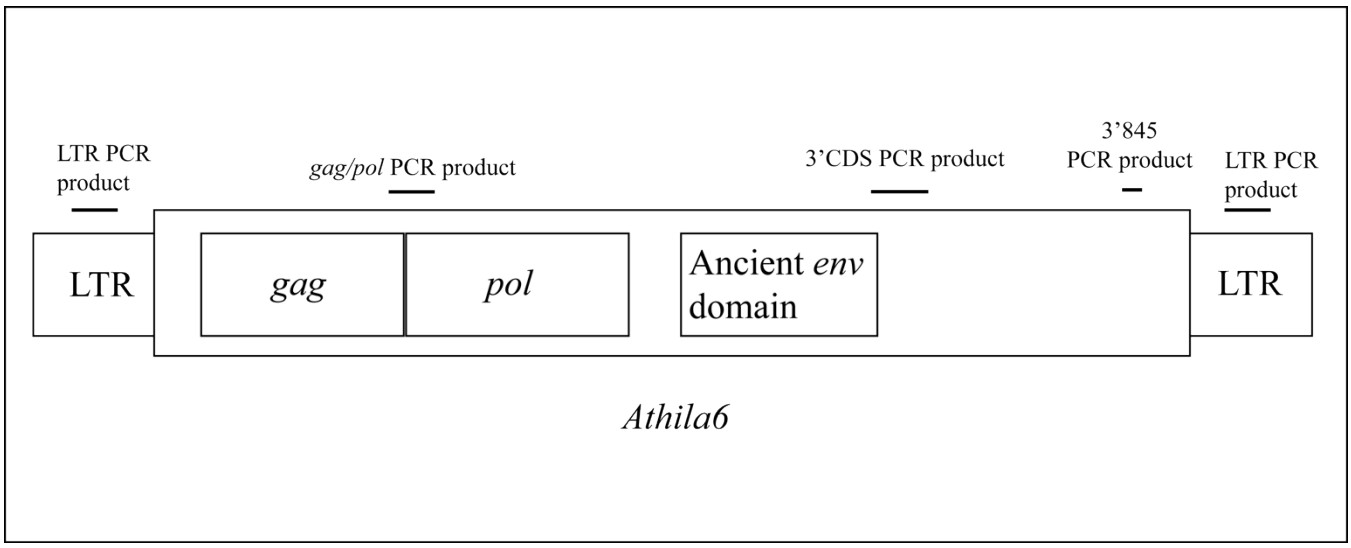

Figure 5 – The structure of the *Athila6* element and the location of the PCR products.

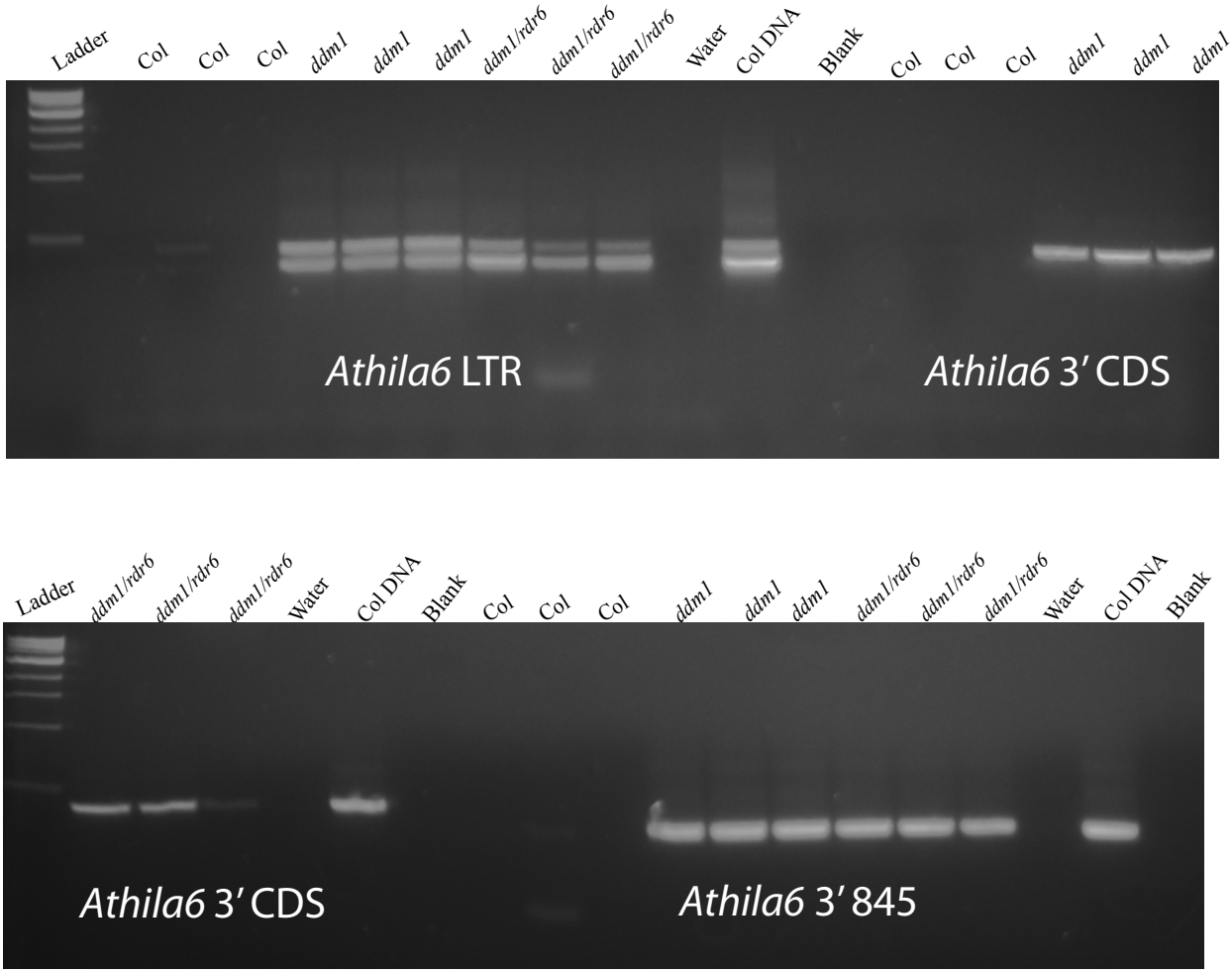

Figure 6 - The PCR products of the *Athila6* LTR, *Athila6* 3'CDS and *Athila6* 3' 845 primers. (The agarose gel for the *Athila6* gag/pol is not shown.)

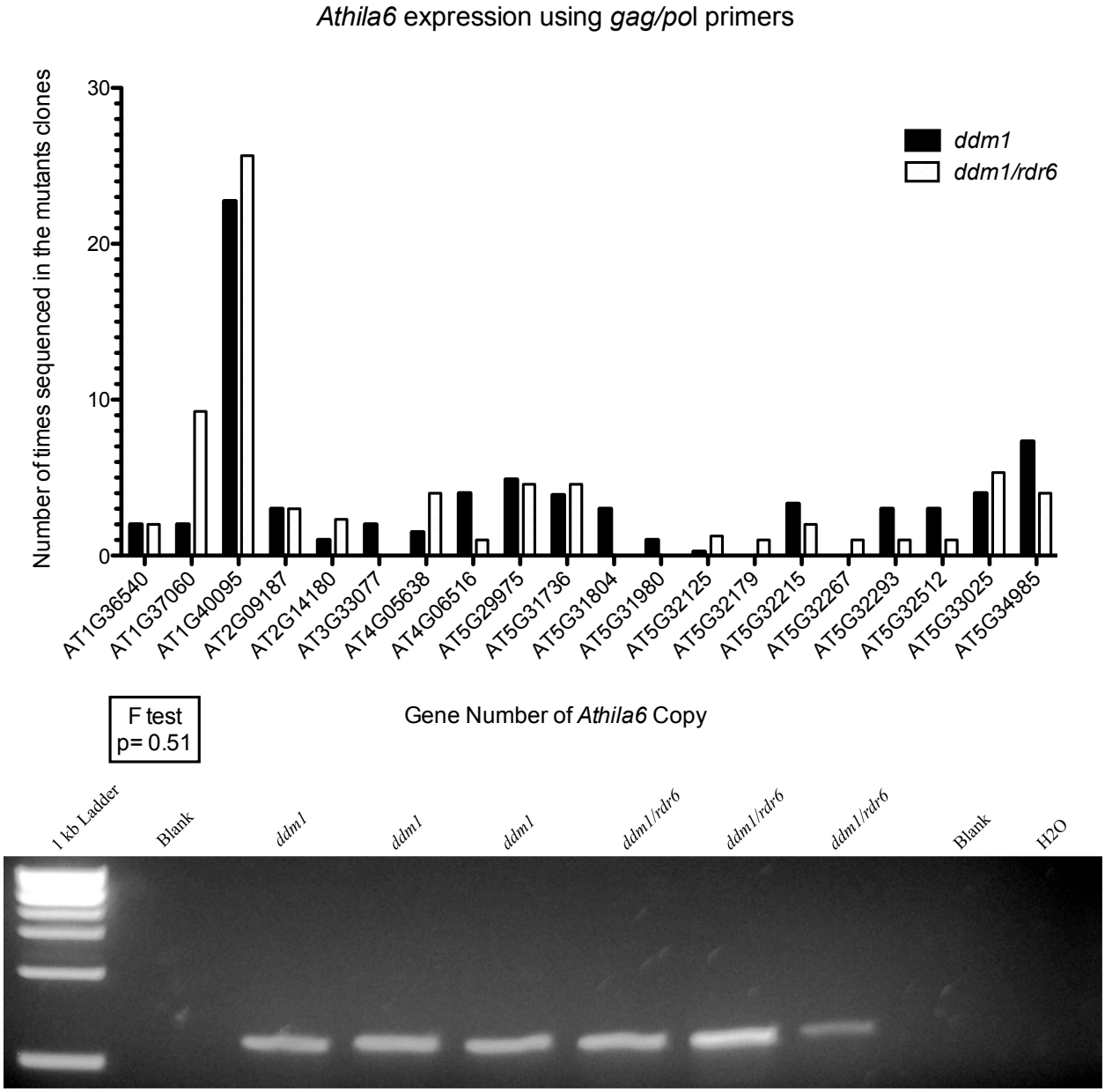

Figure 7- (Top Panel) The gene numbers of the *Athila6* elements sequenced in the *ddm1* clones and the *ddm1/rdr6* clones versus the number of times those elements were sequenced. The F-test analysis of the variances between *ddm1* and *ddm1/rdr6* gave a probability of 0.51, which is not statistically significant. (Bottom Panel) semi-quantitative RT-PCR of the 3 biological replicates of *ddm1* and the 3 replicates of *ddm1/rdr6* using a primer specific to *Athila6* element AT1G37060. It was sequenced more times in *ddm1/rdr6* clones (Top panel) but the amount of RT-PCR product looks equal between *ddm1* and *ddm1/rdr6*.

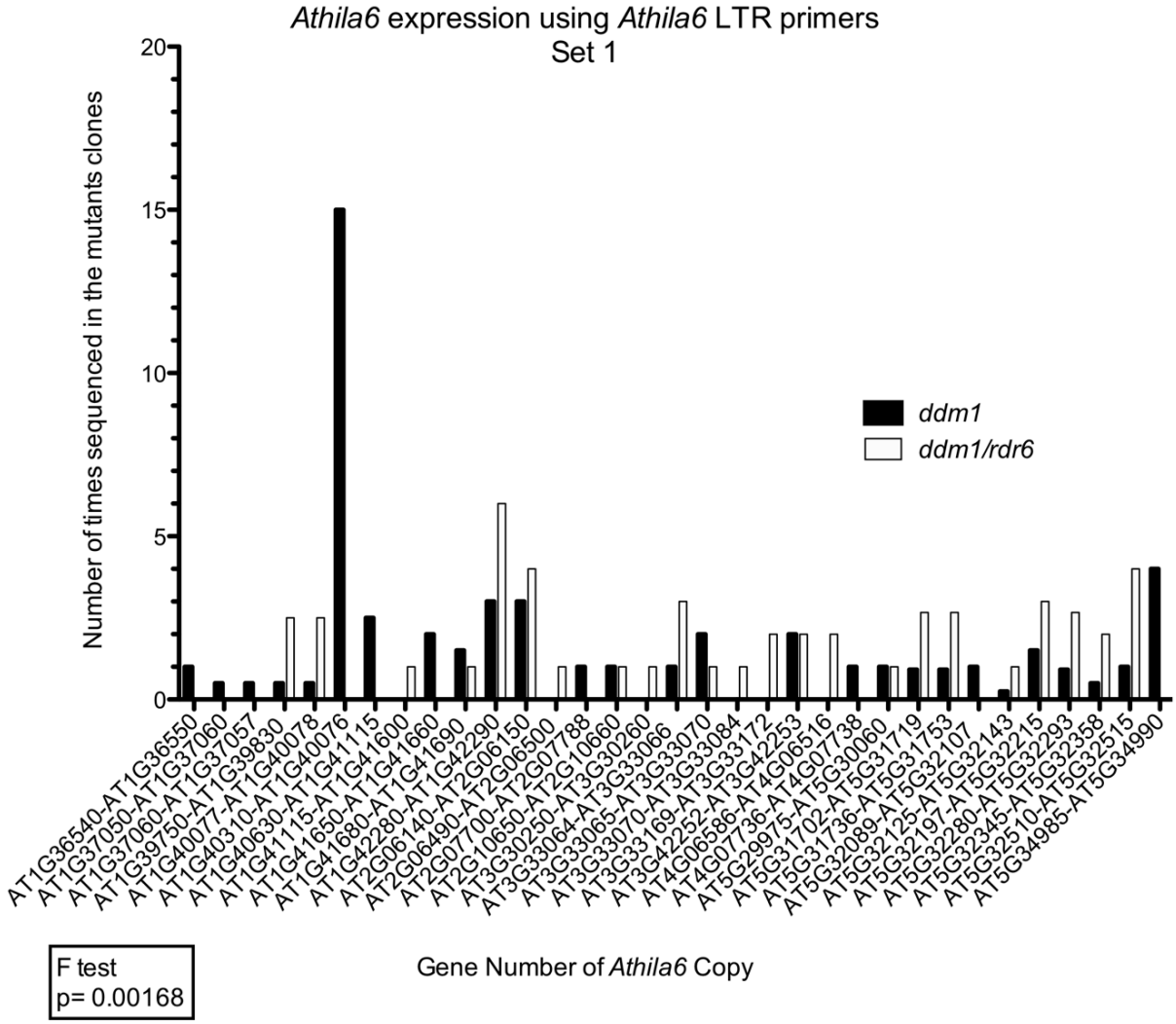

Figure 8 - The gene numbers of the *Athila6* elements sequenced in the *ddm1* clones and the *ddm1/rdr6* clones versus the number of times those elements were sequenced. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.00168 which is statistically significant meaning the difference in variances between the two mutants are not due only to random chance.

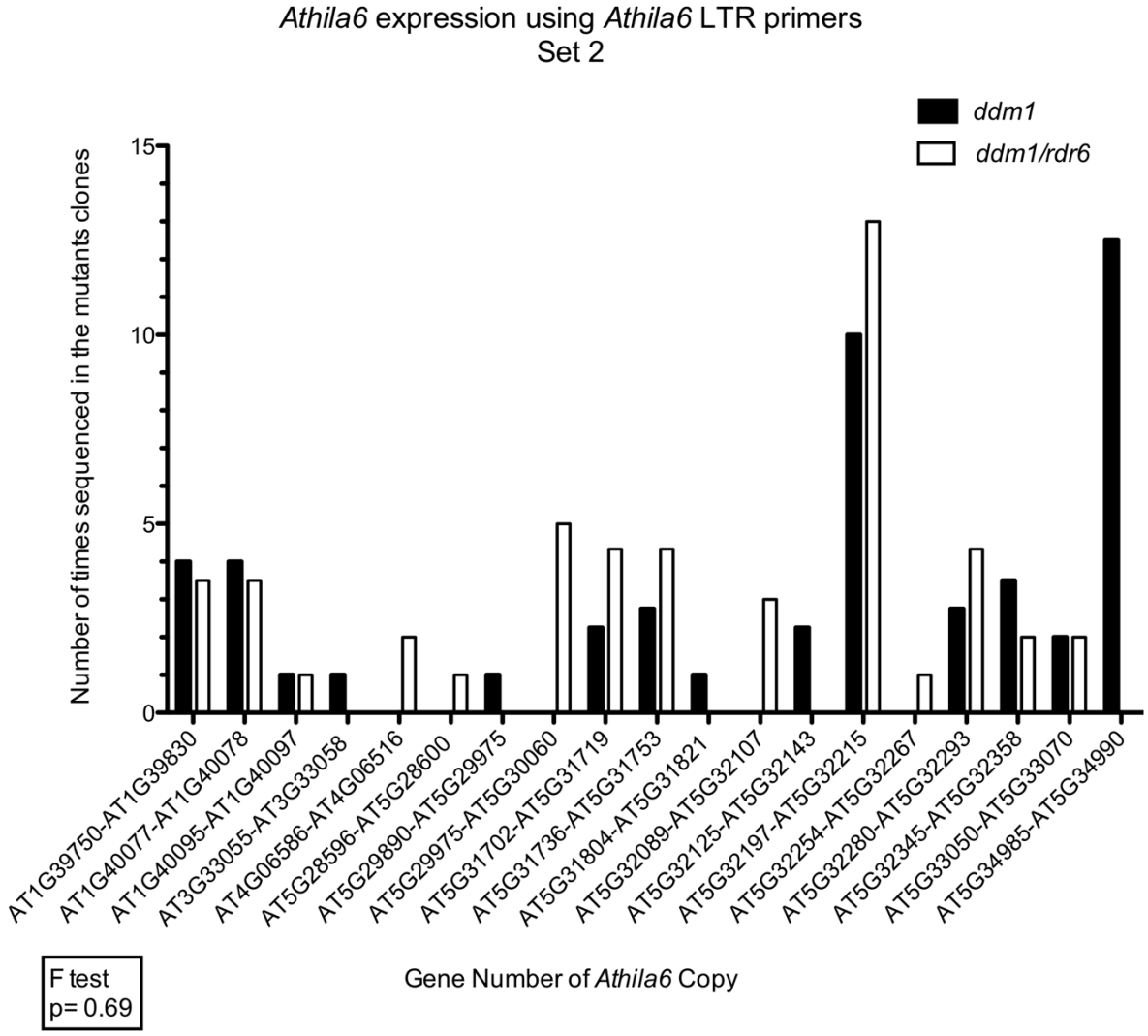

Figure 9- The gene numbers of the *Athila6* elements sequenced in the *ddm1* clones and the *ddm1/rdr6* clones versus the number of times those elements were sequenced. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a not statically significant probability of 0.69 meaning the difference variances between the two mutants are due to random chance.

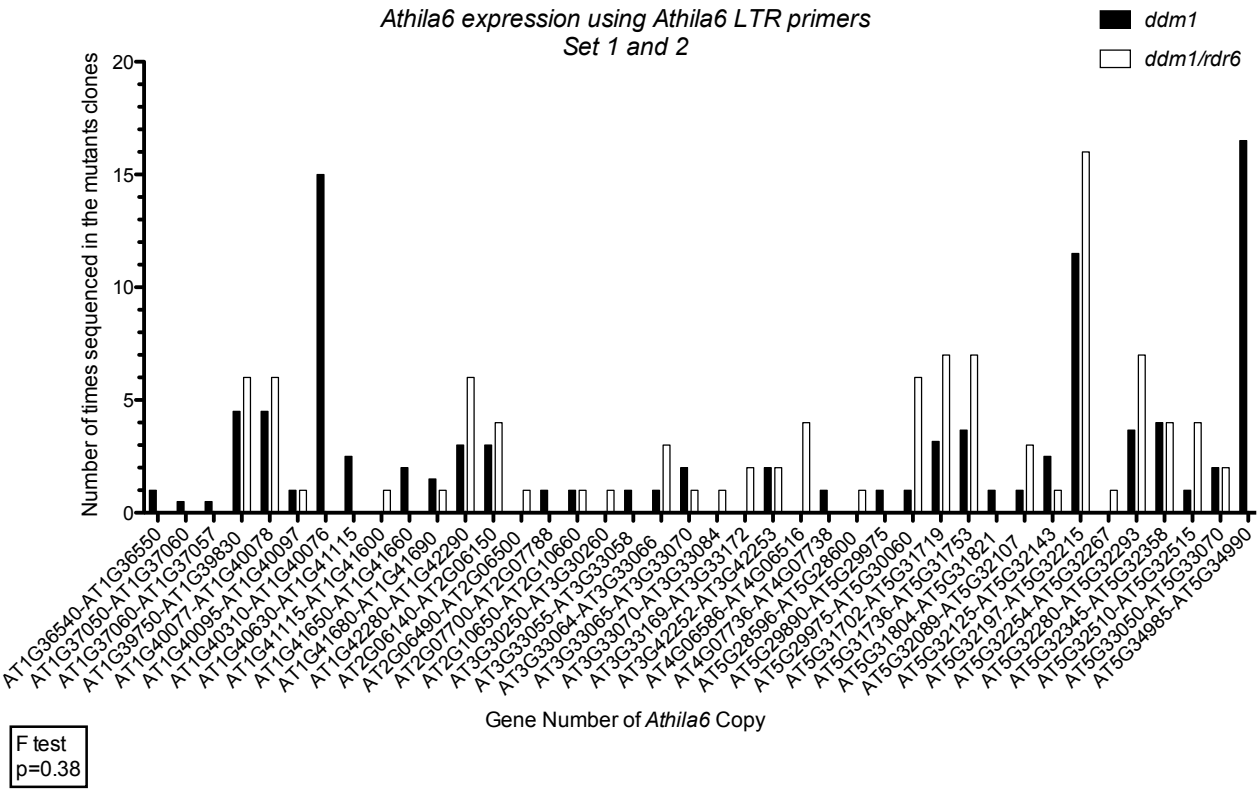

Figure 10- The gene numbers of all the *Athila6* elements sequenced in the *ddm1* clones and the *ddm1/rdr6* clones in both *Athila6* LTR primer sets versus combined sum of the number times those elements were sequenced in those primer sets. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.38 meaning the overall difference in variances between the two mutants are not statistically significant in the *Athila6* LTR primer set and that the differences in variances are due to random chance. However, it seems that there are two LTRs that are expressed more in ddm1 than ddm1/rdr6.

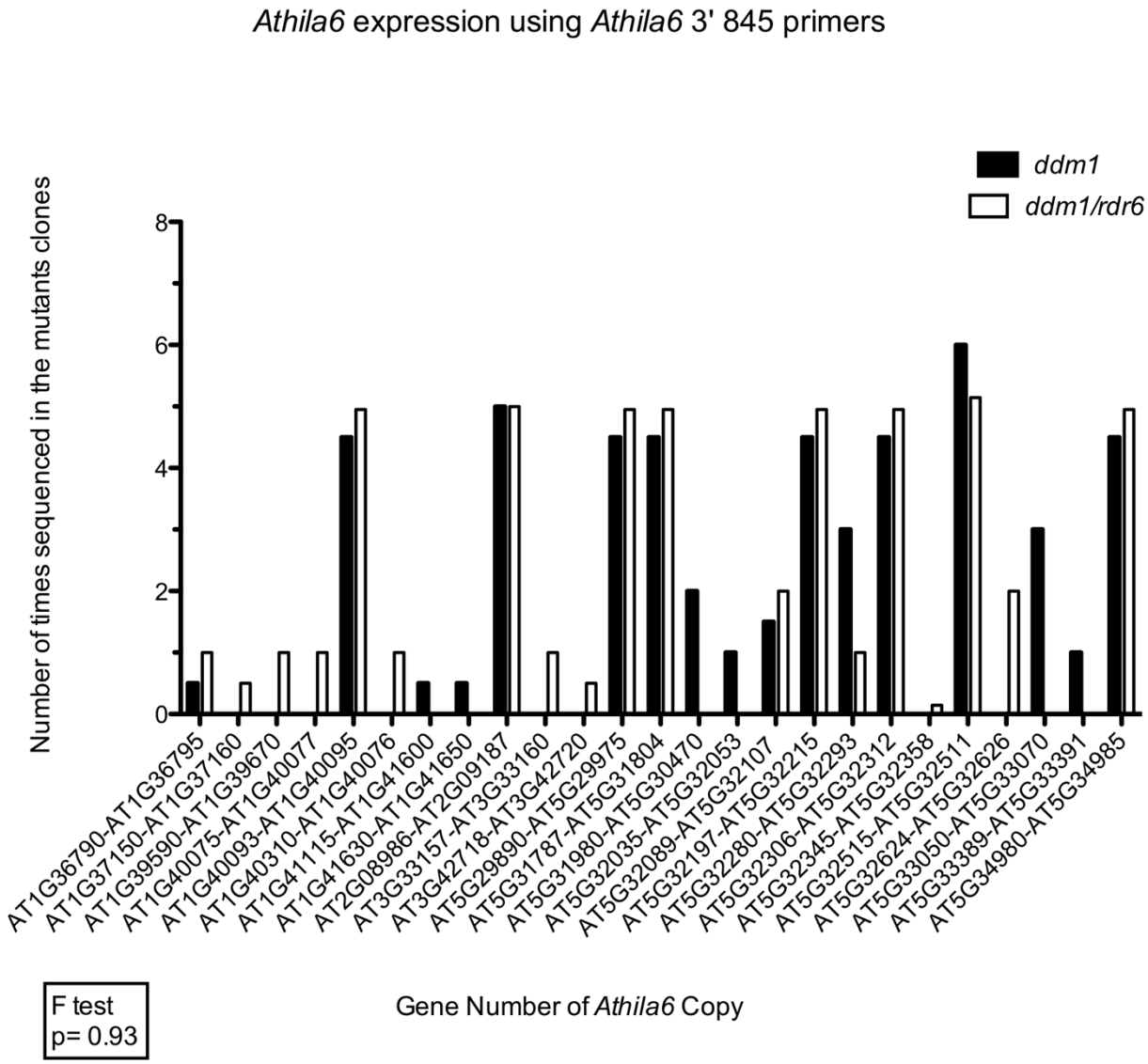

Figure 11- The gene numbers of the *Athila6* elements sequenced in the *ddm1* clones and the *ddm1/rdr6* clones versus the number of times those elements were sequenced. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.93 meaning the difference in variances between the two mutants are not statistically significant and are due to random chance.

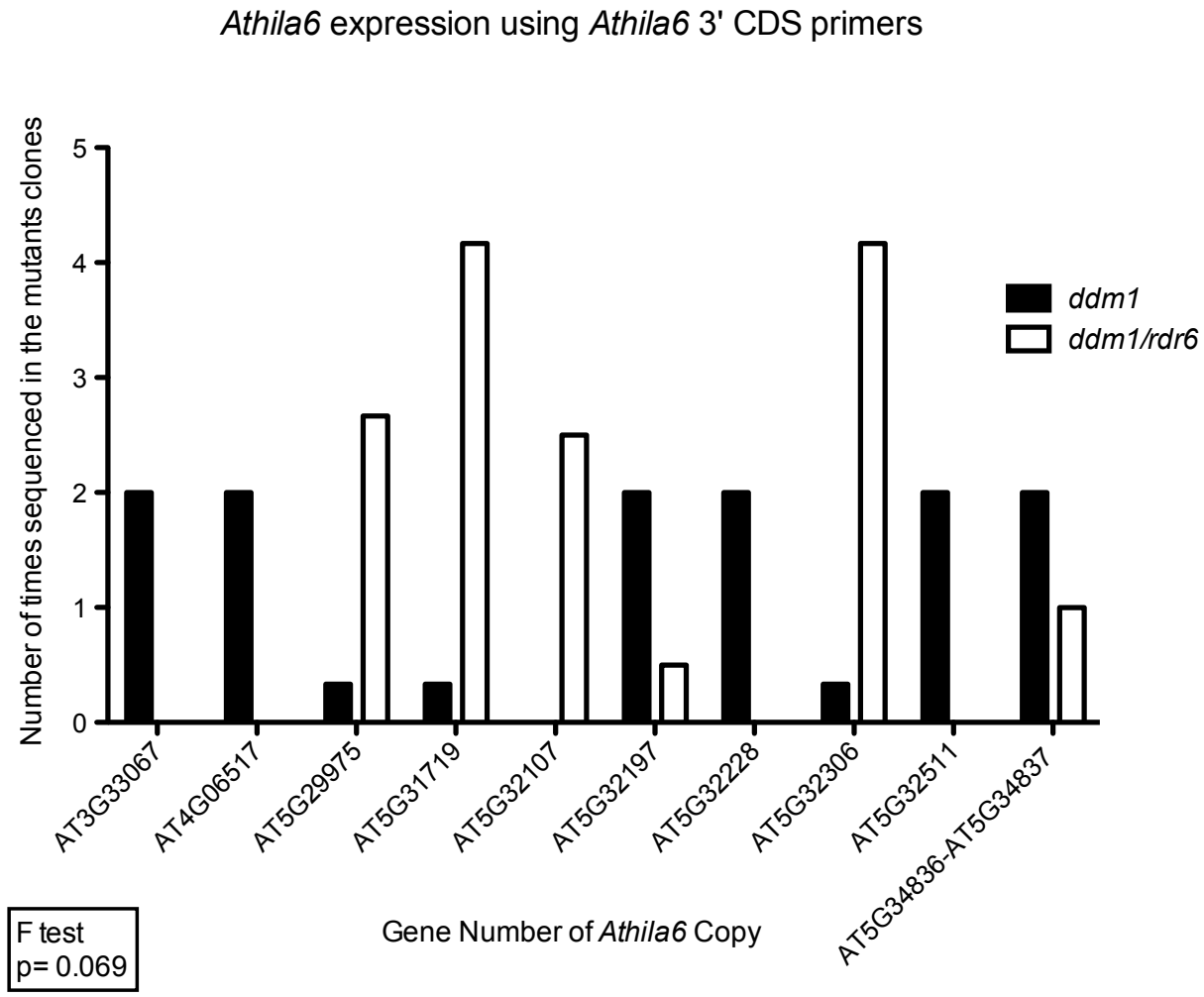

Figure 12- The gene numbers of the *Athila6* elements sequenced in the original *ddm1* clones and the original *ddm1/rdr6* clones versus the number of times those elements were sequenced. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.069 meaning the difference in variances between the two mutants are not statistically significant but this probability is close to 0.05 and there were few clones which necessitating a repeated TOPO cloning.

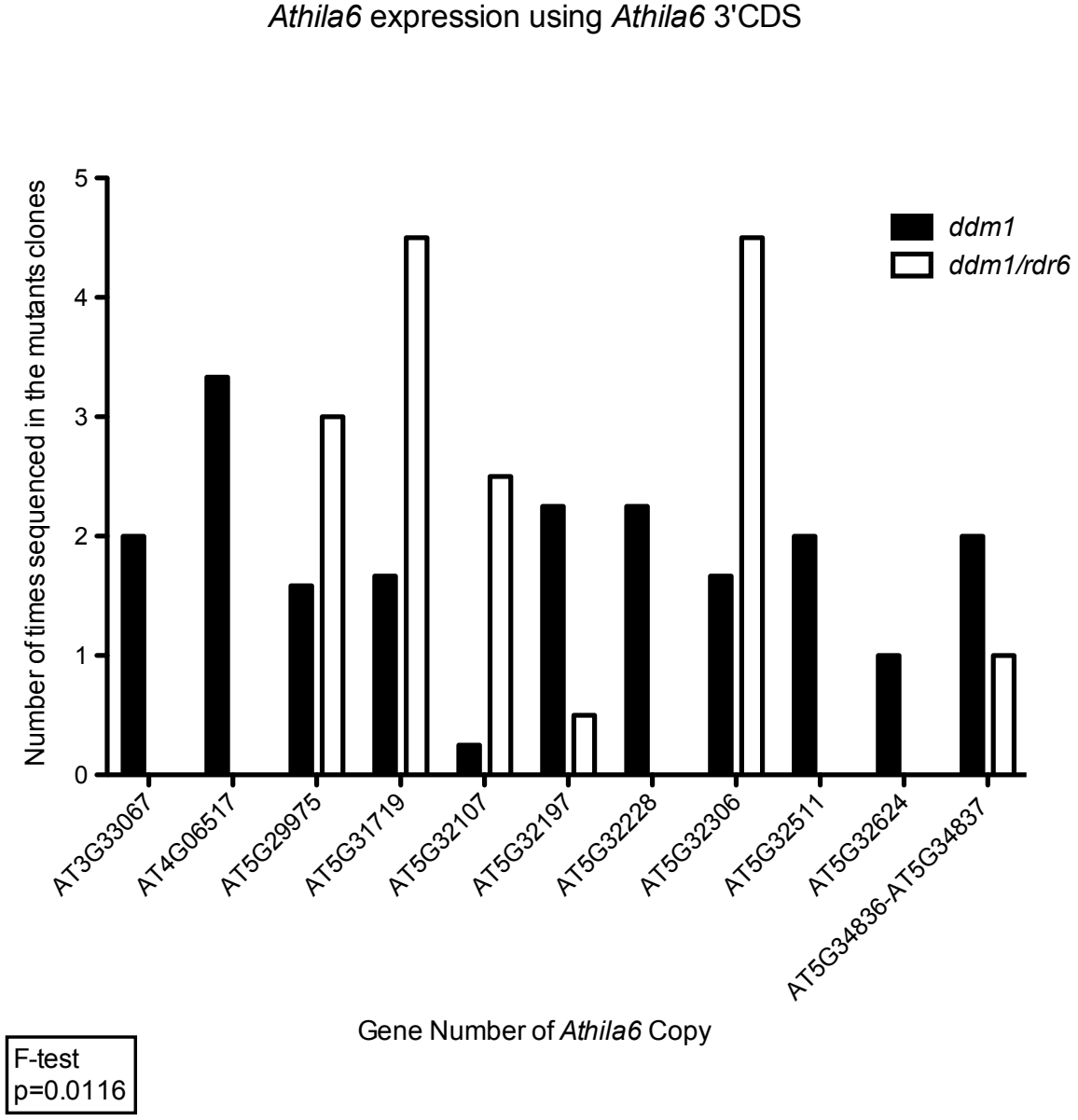

Figure 13- The gene numbers of the *Athila6* elements sequenced in the combined *ddm1* clones and combined *ddm1/rdr6* clones versus the combined total of the number of times those elements were sequenced in the original and repeated TOPO cloning. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.0116 meaning the difference in variances between the two mutants are statistically significant and not due to random chance.

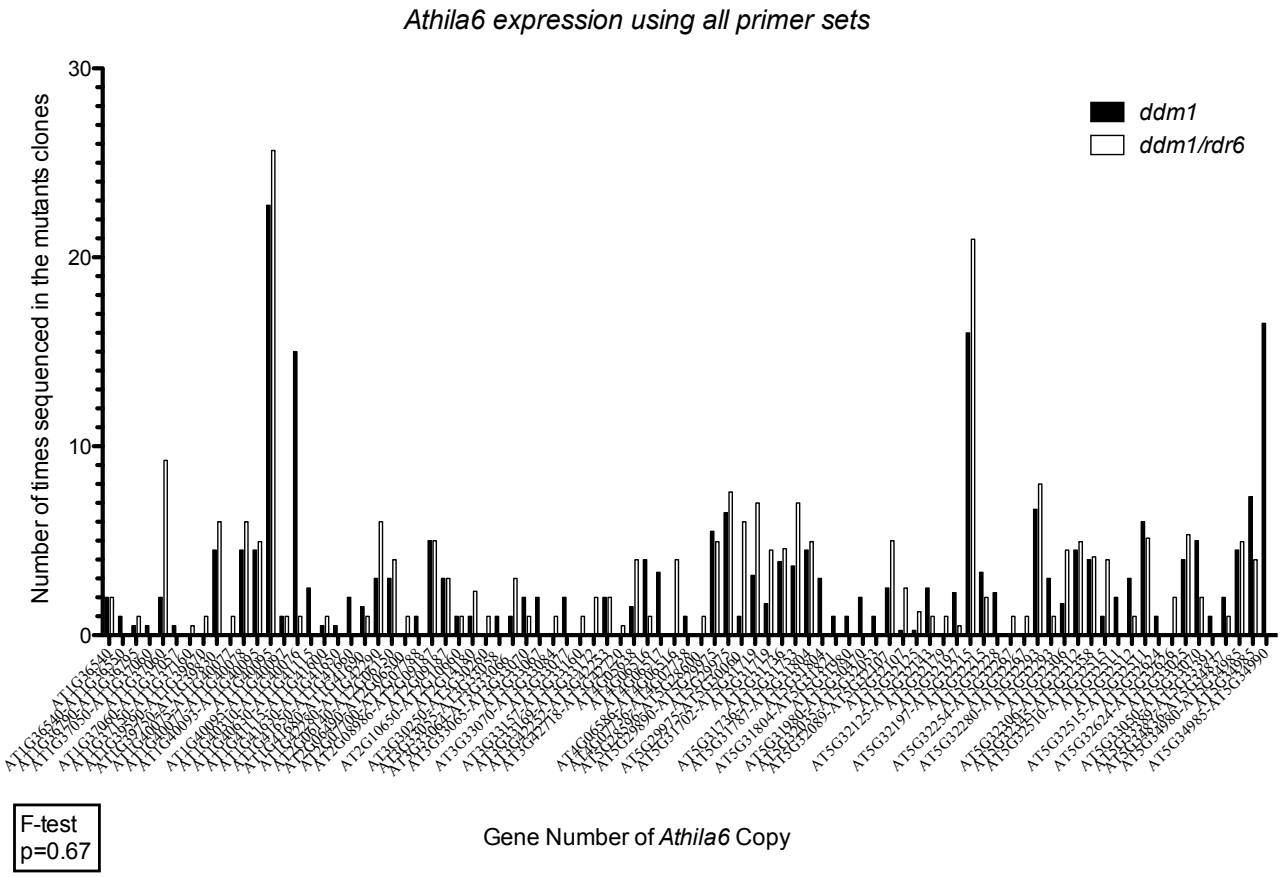

Figure 14- The gene numbers of the *Athila6* elements sequenced in the combined *ddm1* clones and *ddm1/rdr6* clones versus the combined total of the number of times those elements were sequenced in all the primer sets. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.67 meaning the difference in variances between the two mutants are not statistically significant are caused by random chance.

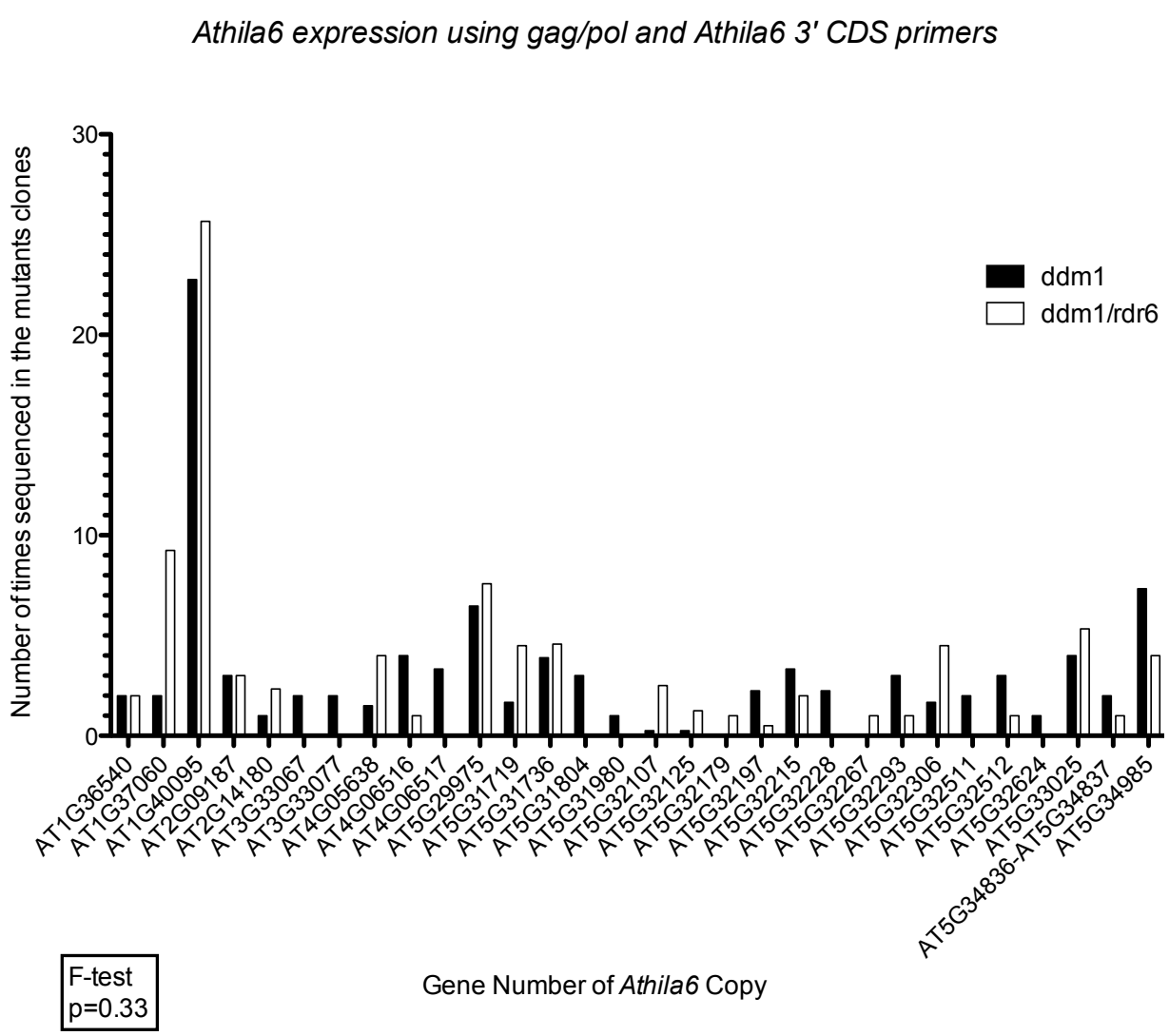

Figure 15- The gene numbers of the *Athila6* elements sequenced in the combined genic *ddm1* and *ddm1/rdr6* clones versus the combined total of the number of times those elements were sequenced in the *gag/pol* and *Athila6* 3'CDS primer sets. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.33 meaning the difference in variances between the two mutants in the genic region are not statistically significant and caused by random chance.

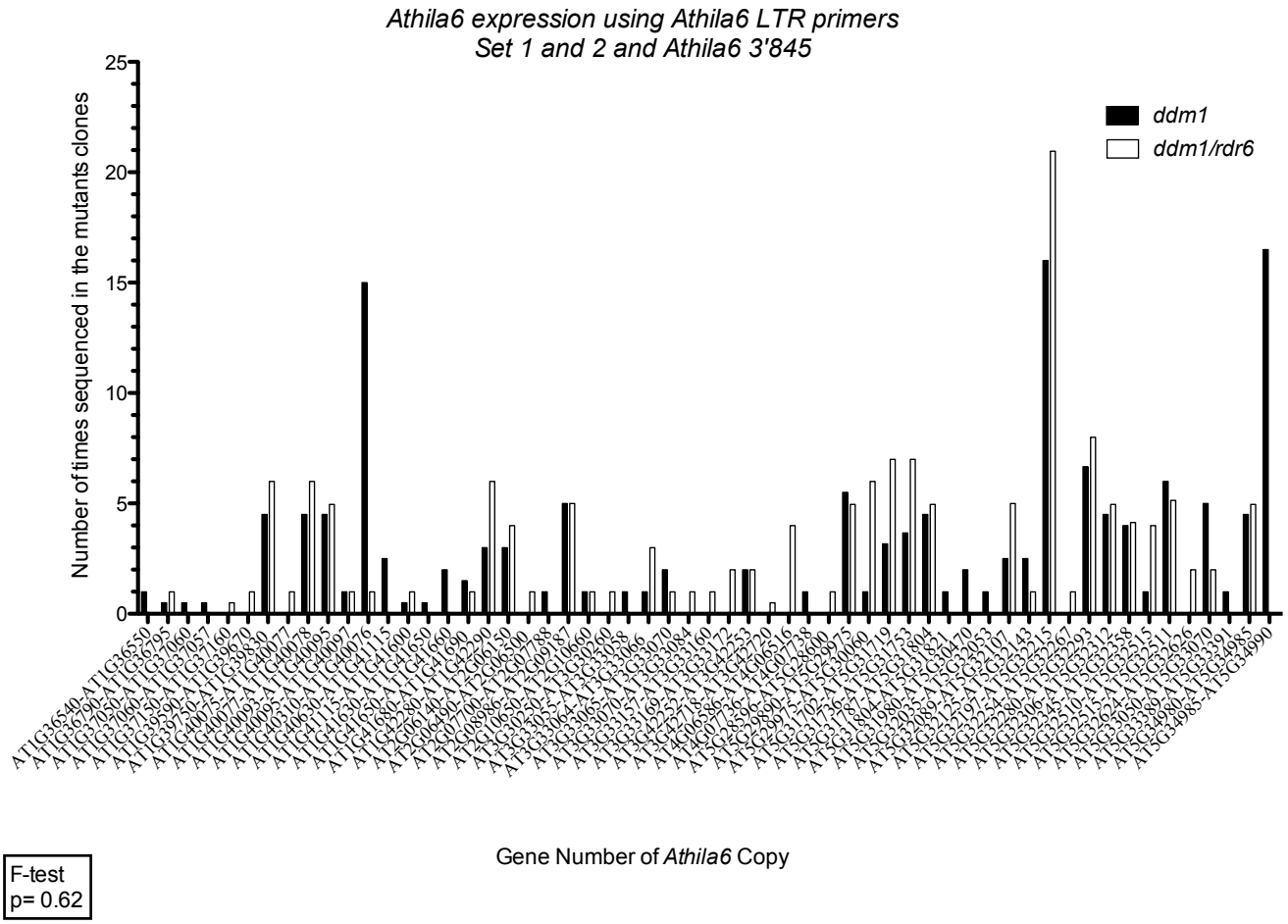

Figure 16- The gene numbers of the *Athila6* elements sequenced in the combined intergenic *ddm1* and *ddm1/rdr6* clones versus the combined total of the number of times those elements were sequences in the *Athila6* LTR sets 1and 2 and *Athila6* 3'845 primer set. The F-test analysis of the variance between *ddm1* and *ddm1/rdr6* gave a probability of 0.62 meaning the difference in variances between the two mutants in the intergenic region are not statistically significant and due to random chance.

Gene Number	*ddm1*	*ddm1/rdr6*
AT1G36540-AT1G36550	1	0
AT1G37050-AT1G37060	0.5	0
AT1G37060-AT1G37057	0.5	0
AT1G37150-AT1G37160	0	0.5
AT1G39590-AT1G39670	0	1
AT1G40075-AT1G40077	0	1
AT1G40630-AT1G41115	2.5	0
AT1G41630-AT1G41650	0.5	0
AT1G41650-AT1G41660	2	0
AT2G06490-AT2G06500	0	1
AT2G07700-AT2G07788	1	0
AT3G30250-AT3G30260	0	1
AT3G33055-AT3G33058	1	0
AT3G33067	2	0
AT3G33070-AT3G33084	0	1
AT3G33077	2	0
AT3G33157-AT3G33160	0	1
AT3G33169-AT3G33172	0	2
AT3G42718-AT3G42720	0	0.5
AT4G06517	3.333333333	0
AT4G06586-AT4G06516	0	4
AT4G07736-AT4G07738	1	0
AT5G28596-AT5G28600	0	1
AT5G31804	3	0
AT5G31804-AT5G31821	1	0
AT5G31980	1	0
AT5G31980-AT5G30470	2	0
AT5G32035-AT5G32053	1	0
AT5G32179	0	1
AT5G32228	2.25	0
AT5G32254-AT5G32267	0	1
AT5G32267	0	1
AT5G32511	2	0
AT5G32624	1	0
AT5G32624-AT5G32626	0	2
AT5G33389-AT5G33391	1	0
AT5G34985-AT5G34990	16.5	0
Total	48.08333333	19
	22 in *ddm1* only	15 in *ddm1/rdr6* only

Figure 17- A list of all the *Athila6* elements unique to one of the mutants as well as the number of times that element was sequenced in the mutants. The elements unique to *ddm1* are highlighted in red, and the ones unique to *ddm1/rdr6* are highlighted in blue. There is a greater number and abundance of elements unique to *ddm1* than there is to *ddm1/rdr6*.

Set Number	Forward Primer	Reverse Primer	PCR Product Size	Difference between *ddm1* and *ddm1/rdr6*
1	*Athila6* 5' LTR in F	*Athila6* LTR R	546 bp	No
2	*Athila6* 5' LTR in F	*Athila6* CDS R	1997 bp	No
3	*Athila6* 5' LTR in F	*Athila6* 5' env R	5726 bp	Yes
4	*Athila6* 5' LTR in F	*Athila6* 3' CDS R	6344 bp	No
5	*Athila6* 5' LTR in F	*Athila6* entire 3' R	8431 bp	No
6	*Athila6* LTR F	*Athila6* LTR R	446 bp	No
7	*Athila6* LTR F	*Athila6* CDS R	1897 bp	No
8	*Athila6* LTR F	*Athila6* 5' env R	5626 bp	No
9	*Athila6* LTR F	*Athila6* 3' CDS R	6244 bp	No
10	*Athila6* LTR F	*Athila6* entire 3' R	8331 bp	No
11	*Athila6* LTR F	*Athila6* LTR R Outer	8570 bp	Yes
12	*Athila6* CDS F	*Athila6* CDS R	476 bp	No
13	*Athila6* CDS F	*Athila6* 5' env R	4205 bp	Yes
14	*Athila6* CDS F	*Athila6* 3' CDS R	4823 bp	No
15	*Athila6* CDS F	*Athila6* entire 3' R	6910 bp	Yes
16	*Athila6* CDS F	*Athila6* LTR R Outer	7149 bp	No
17	*Athila6* CDS F	*Athila6* LTR R	8748 bp	Yes
18	*Athila6* 3' CDS F	*Athila6* 3' CDS R	422 bp	No
19	*Athila6* 3' CDS F	*Athila6* entire 3' R	2509 bp	No
20	*Athila6* 3' CDS F	*Athila6* LTR R Outer	2748 bp	Yes
21	*Athila6* 3' CDS F	*Athila6* LTR R	4347 bp	Yes
22	*Athila6A* entire 3'F	*Athila6* 5' env R	794 bp	No
23	*Athila6A* entire 3'F	*Athila6* 3' CDS R	1412 bp	No
24	*Athila6A* entire 3'F	*Athila6* entire 3' R	3499 bp	No
25	*Athila6A* entire 3'F	*Athila6* LTR R Outer	3738 bp	No
26	*Athila6A* entire 3'F	*Athila6* LTR R	5337 bp	No

Figure 18- The different combinations of PCR primers used, the size of the PCR product and if there was a substantial difference between *ddm1* and *ddm1/rdr6*. Sets 3,11,13,15,17, 20 and 21 had substantial differences between the two mutants.

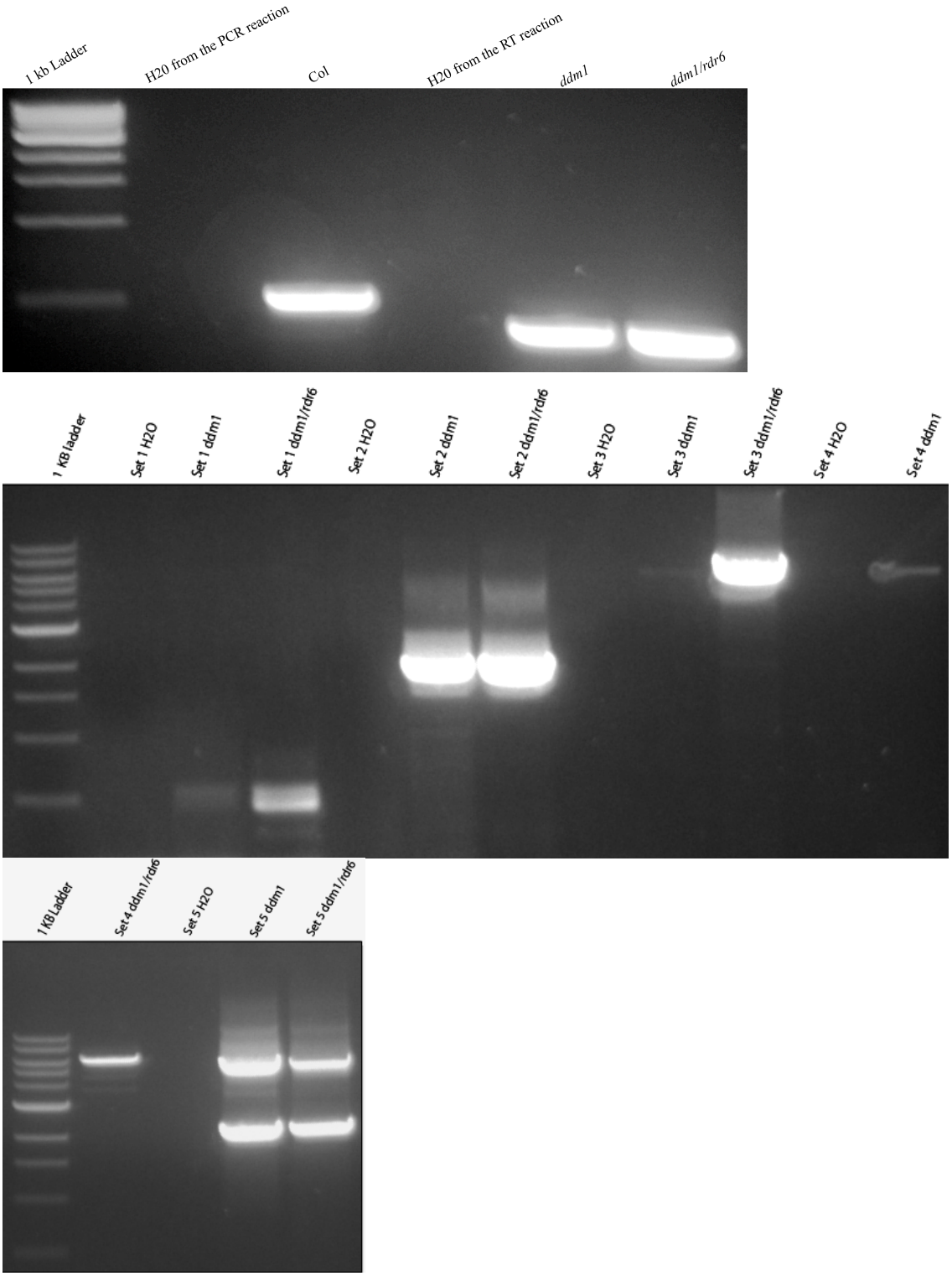

Figure 19- (Top panel) Control PCR using the constitutively expressed gene tyrAT. The *ddm1* and *ddm1/*rdr6 PCR product is smaller than the Col product, which means, the *ddm1* and *ddm1/rdr6* cDNA is free of DNA contamination. (Bottom panels) The PCR products from sets 1 through 5. PCR product of *ddm1/rdr6* in set 3 is considerably brighter than its *ddm1* counter part. Set 5 has no difference in the mutants but does have two PCR products when there should have been only one.

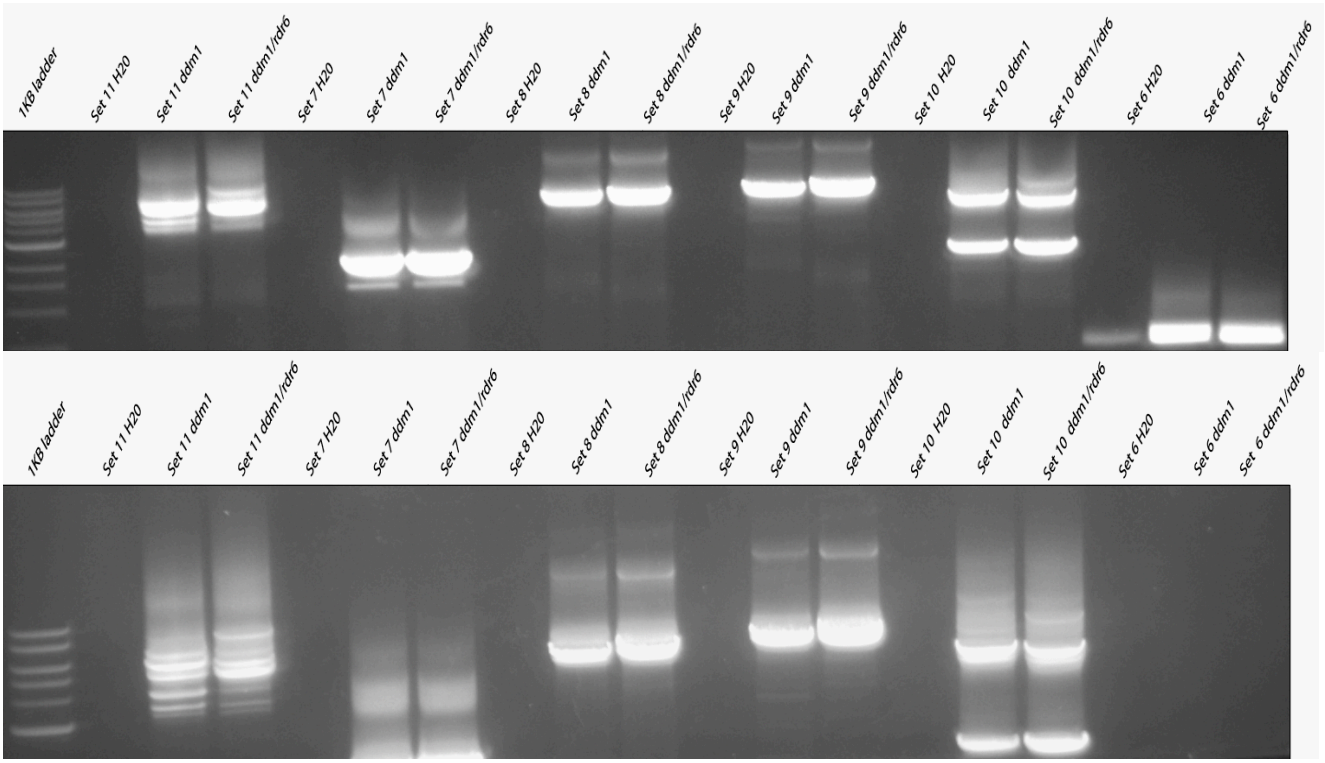

Figure 20- The PCR products from sets 6 through 11. The products were run on the agarose gel again after the top picture was taken in order to separate the top PCR product unique to *ddm1/rdr6* from the rest of the set 11's PCR products. After, the top PCR product in set 11 was separated from the rest of set 11's PCR products, as seen in the bottom picture, it was excised from the gel and cloned.

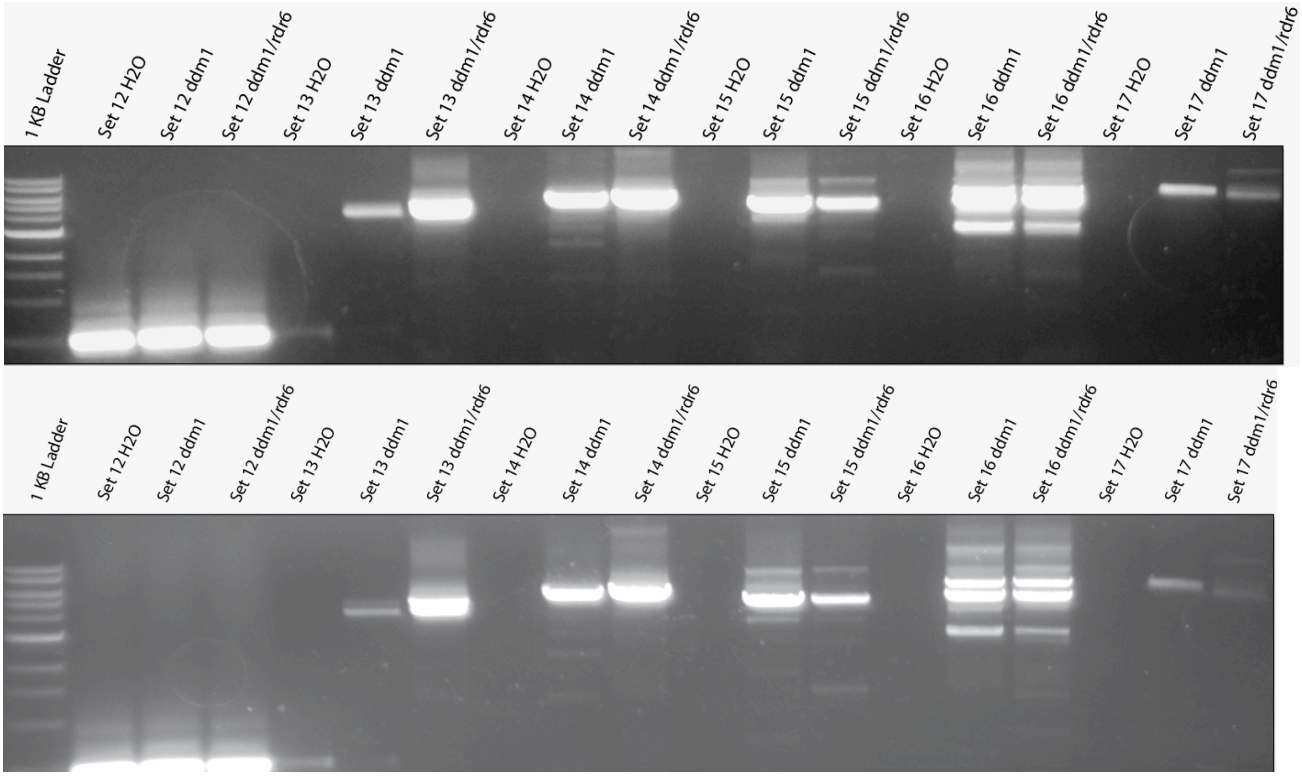

Figure 21- The PCR products from sets 12 through 17. The products were run on the agarose gel again after the top picture was taken in order to separate some of the PCR products in set 16. The additional PCR products in sets 15 and 17 were too faint to clone. Though PCR product for *ddm1/rdr6* in set 13 was cloned. It was then discovered that its one bright PCR product was actually in fact two PCR products.

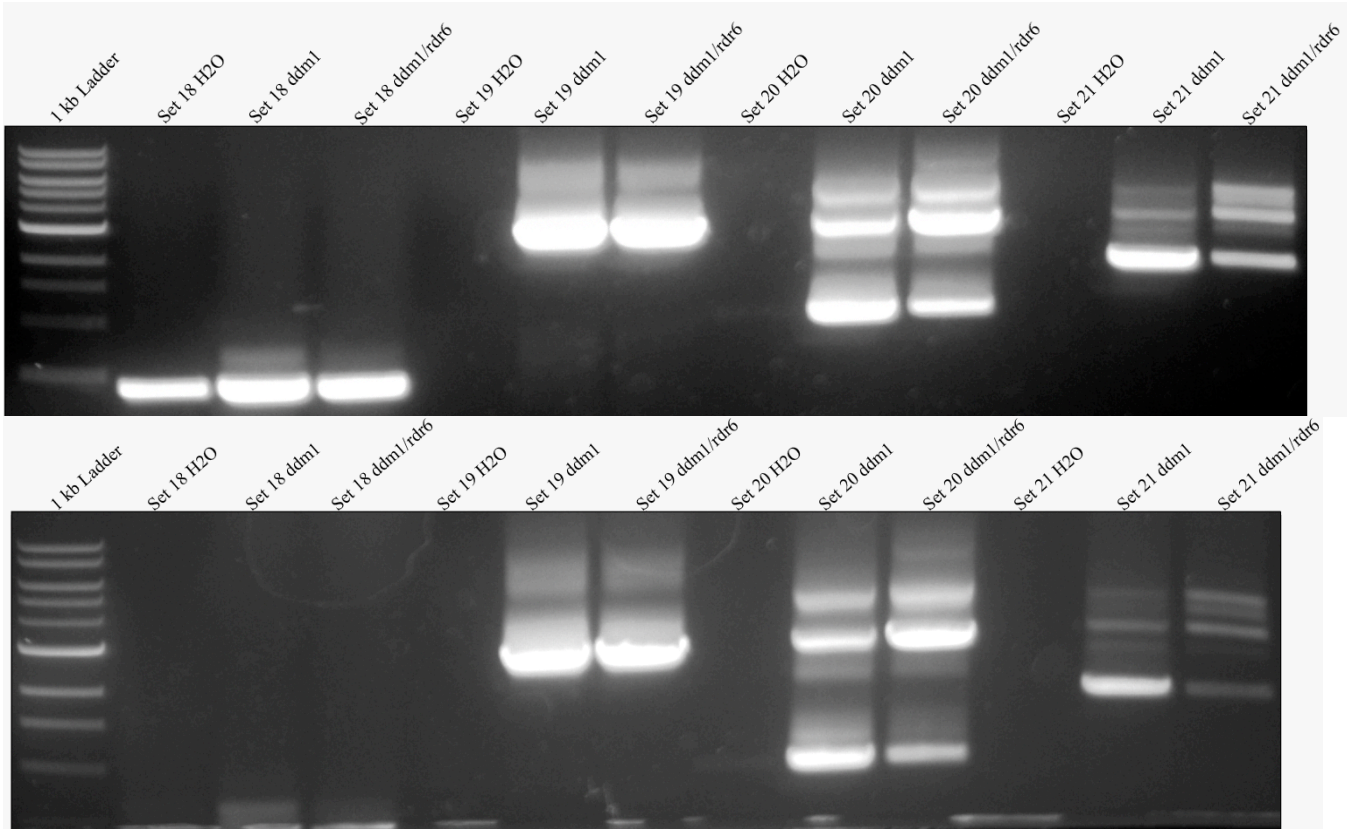

Figure 22- The PCR products from sets 18 through 21. The products were run on the agarose gel again after the top picture was taken in order to separate the PCR products in sets 20 and 21. The additional PCR products of *ddm1/rdr6* found in sets 20 and 21 were too faint to clone.

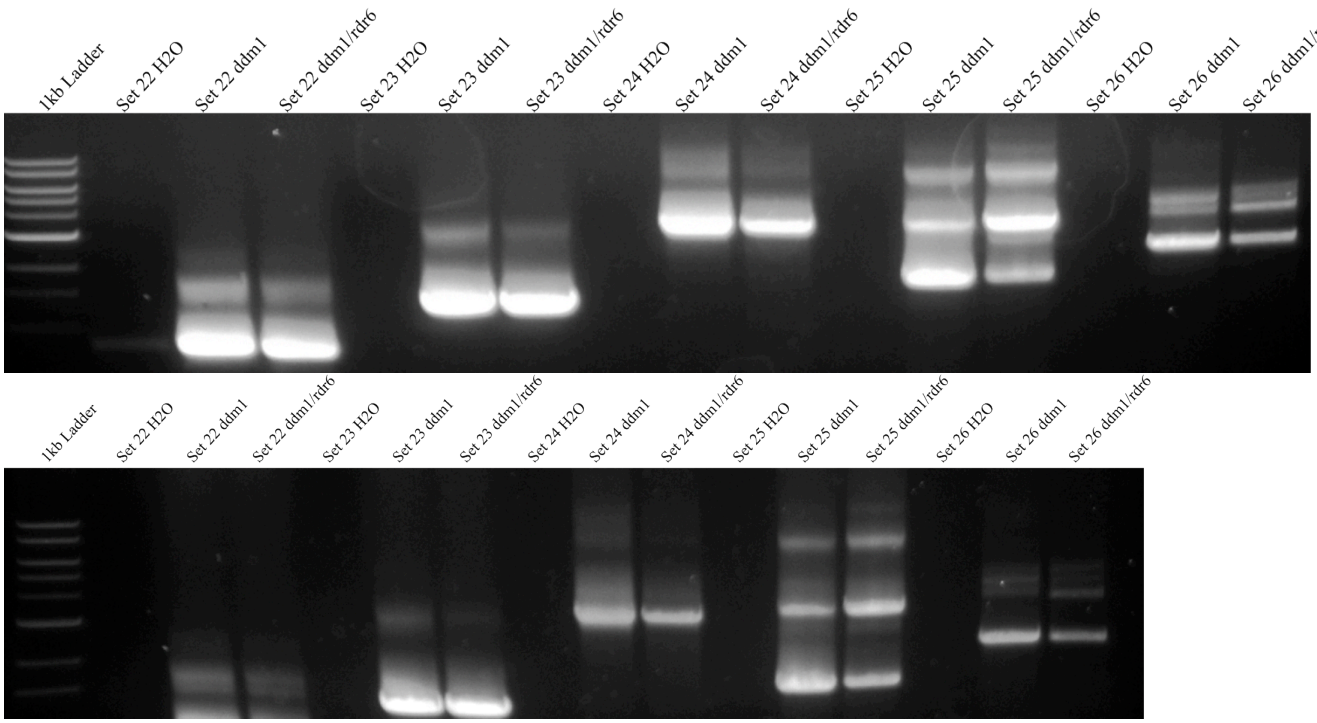

Figure 23- The PCR products from sets 22 through 26. The products were run on the agarose gel again after the top picture was taken in order to separate the PCR products in set 26. No additional PCR products of *ddm1/rdr6* or other differences in *ddm1/rdr6* when compared to *ddm1* were found in sets 22 through 26.

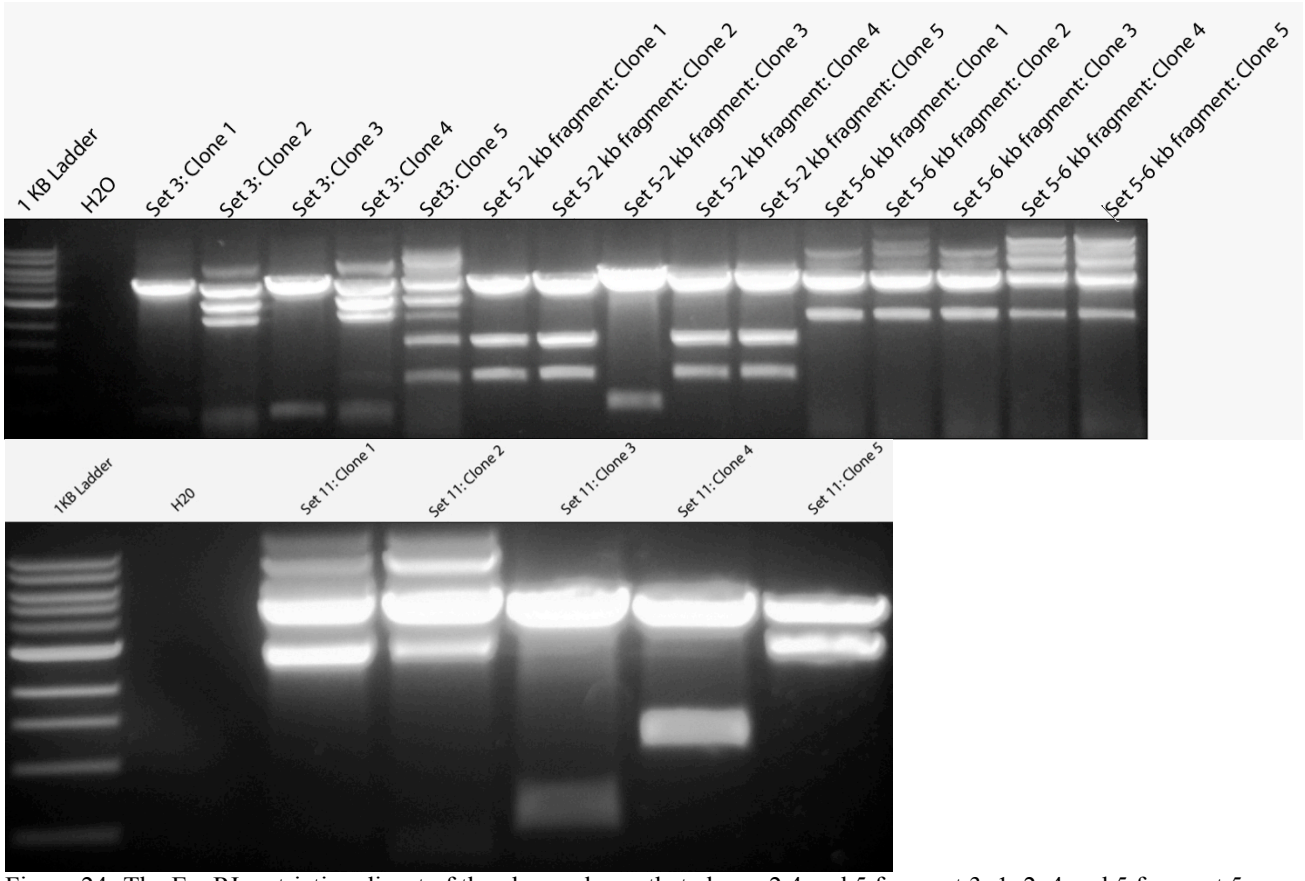

Figure 24- The EcoRI restriction digest of the clones shows that clones 2,4 and 5 from set 3; 1, 2, 4 and 5 from set 5, the 2 kb fragment; 1, 2, 3, 4 and 5 from set 5, the 6 kb fragment; and 1, 2, 3, 4 and 5 from set 11 all have an insert corresponding to their respective PCR products.